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# $PLA_2$ Inhibitors of Angiogenesis

## FIELD OF THE INVENTION

The present invention relates to the treatment of diseases, in a mammal, in which inappropriate, excessive or undesirable angiogenesis has occurred.

## BACKGROUND OF THE INVENTION

Chronic diseases are often accompanied by profound angiogenesis, which can contribute to or maintain an inflammatory and/or proliferative state, or which leads to tissue destruction through the invasive proliferation of blood vessels. IL-1β and TNFα have been found to be potent inducers of angiogenesis *in vivo* and are commonly involved in the pathology of chronic inflammatory diseases (Folkman and Shing, J. Biol. Chem. 267:10931, 1992).

Angiogenesis is generally used to describe the development of new or replacement blood vessels, or neovascularisation. It is a necessary and physiologically normal process by which the vasculature is established in the embryo. Angiogenesis does not occur, in general, in the normal adult. Many diseases, however, are characterized by persistent and unregulated angiogenesis. For instance, in arthritis, new capillary blood vessels invade the joint and destroy cartilage (Colville-Nash and Scott, Ann. Rheum. Dis., 51, 919,1992). In diabetes, new vessels invade the vitreous and blood, and cause blindness (and may occur in many different eye diseases) (Brooks et al., Cell, 79, 1157, 1994). The process of atherosclerosis has been linked to angiogenesis (Kahlon et al., Can. J. Cardiol. 8, 60, 1992). Tumor growth and metasis have been found to be angiogenesis-dependent (Folkman, Cancer Biol, 3, 65, 1992; Denekamp, Br. J. Rad. 66, 181, 1993; Fidler and Ellis, Cell, 79, 185, 1994).

The recognition of the involvement of angiogenesis in major diseases has been accompanied by research to identify and develop inhibitors of angiogenesis. These inhibitors are generally classified in response to discrete targets in the angiogenesis cascade, such as activation of endothelial cells by an angiogenic signal; synthesis and release of degradative enzymes; endothelial cell migration; proliferation of endothelial cells; and formation of capillary tubules. Therefore,

angiogenesis occurs in many stages and attempts are underway to discover and develop compounds that work to block angiogenesis at these various stages.

There are publications that teach that inhibitors of angiogenesis, working by diverse mechanisms, are beneficial in diseases such as cancer and metastasis (O'Reilly et al., Cell, 79, 315, 1994; Ingber et al., Nature, 348, 555, 1990), ocular diseases (Friedlander et al., Science, 270, 1500, 1995), arthritis (Peacock et al., J. Exp. Med. 175, 1135, 1992; Peacock et al., Cell. Immun. 160, 178, 1995) and hemangioma (Taraboletti et al., J. Natl. Cancer Inst. 87, 293, 1995).

A need still exists, however, to find suitable small molecule inhibitors which will block angiogenesis in a mammal for treatment of these diseases which have an angiogenic component. The current application teaches the novel finding that compounds that block cytokine production, can block angiogenesis by removing the early cytokine signals for angiogenesis.

## SUMMARY OF THE INVENTION

The present invention is to the novel use of a $PLA_2$ inhibitor for the treatment of chronic inflammatory or proliferative or angiogenic diseases which are caused by excessive, inappropriate angiogenesis.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure I A and B show the effect of Compound I in vascular casting model of angiogenesis, with IA showing vascular index, and IB showing granuloma dry weight.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention is to the novel use of a $PLA_2$ inhibitor, in particular that of low molecular weight, 14 kDa $PLA_2$, for the treatment of chronic inflammatory diseases which are caused by excessive or inappropriate angiogenesis.

Phospholipase $A_2$'s ($PLA_2$ (EC 3.1.1.4)) are responsible for the liberation of arachidonic acid from the sn-2 position of phospholipid. They are thought to play an important role in the pathogenesis of inflammation and possibly in immunological dysfunction, both as a cell associated enzyme as well as an extracellular soluble enzyme. Low molecular weight, mammalian Type II 14 kDa $PLA_2$ has been well characterized and is known to exist in both an extracellular form in inflammatory fluids (Kramer et al., J. Biol. Chem., 264:5768-5775 (1989) and in a cell associated form (Kanda et al., Biochemical and Biophysical Research Communications, 163:42-

48 (1989) and has been found in a variety of cells and tissues or extracellularly when released in response to antigenic activators or pro-inflammatory mediators such as Interleukin (IL)-1, IL-6 or tumor necrosis factor (TNF). Its presence in such inflammatory fluids, tissue exudates or serum has therefore implicated Type II-14 kDa-$PLA_2$'s role in inflammation (Vadas, et al., (1985) Life Sci. **36**, 579-587; and Seilhamer, et al., (1989) J. Biol. Chem. 264, 5335-5338). Recently, the elevated serum levels of $PLA_2$ activity during an inflammatory insult has been attributed to cytokine induction of acute phase protein release from liver, of which the 14 kDa-$PLA_2$ is suggested to be a part (Crowl, et al., (1991) J. Biol. Chem. 266, 2647-2651). In addition, soluble $PLA_2$ activity is markedly elevated in the serum and synovial fluid of patients with rheumatoid arthritis (Stefanski et al., J. Biochem. 100:1297-303 (1986). Furthermore, increasing serum $PLA_2$ levels have been shown to positively correlate with clinical severity (Bomalaski and Clark, Arthritis and Rheumat. 36:190-198 (1993)). Various inhibitors of $PLA_2$ have been described in publications and in US Patents. See for instance US Patents 4,959,357; 4,933,365; 5,208,223; 5,208244; Marshall et al., J. Rheumatology 18:1 (1991); Marshall et al., Phospholipase A2, Ed. Pyu Wong, Plenum Press, NY (1990) pages 169-181; Wilkerson, et al., Eur. J. Med. Chem., 26:667, 1991 and Wilkerson, Antiinflammatory Phospholipase $A_2$ Inhibitors, Drugs of the Future, Vol. 15, No. 2 p 139-148(1990). Accordingly, as $PLA_2$ is important in the liberation of arachidoninc acid from phospholipid and may also play a role in the generation of PAF via lysophospholipid formation, inhibition of such an enzyme would be useful for the treatment of disease states caused thereby.

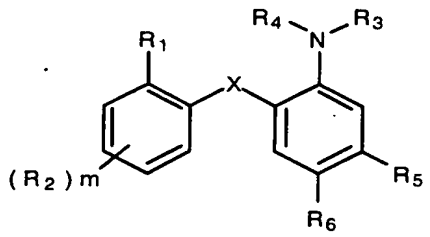
There are many novel forms of phospholipase A2's which have recently been discovered. For the purposes herein, members of the sn-2 acylhydrolase family of PLA2's are divided into low and high molecular weight enzymes be it from mammalian, or non-mammalian sources. Low molecular weight $PLA_2$'s will generally have a molecular weight in the range of 12,000 to 15,000. High molecular weight will be in the range of 30,000 or 56,000 kDa to 110,000 by SDS electrophoresis analysis.

Phospholipase $A_2$'s ($PLA_2$ (EC 3.1.1.4)) are responsible for the liberation of arachidonic acid from the sn-2 position of phospholipid. They are thought to play an important role in the pathogenesis of inflammation and possibly in immunological dysfunction, both as a cell associated enzyme as well as an extracellular soluble enzyme. Low molecular weight, mammalian Type II 14 kDa $PLA_2$ has been well characterized and is known to exist in both an extracellular form in inflammatory

fluids (Kramer et al., J. Biol. Chem., 264:5768-5775 (1989) and in a cell associated form (Kanda et al., Biochemical and Biophysical Research Communications, 163:42-48 (1989) and has been found in a variety of cells and tissues or extracellularly when released in response to antigenic activators or pro-inflammatory mediators such as Interleukin (IL)-1, IL-6 or tumor necrosis factor (TNF). Its presence in such inflammatory fluids, tissue exudates or serum has therefore implicated Type II-14 kDa-$PLA_2$'s role in inflammation (Vadas, et al., (1985) Life Sci. **36**, 579-587; and Seilhamer, et al., (1989) J. Biol. Chem. 264, 5335-5338). Recently, the elevated serum levels of $PLA_2$ activity during an inflammatory insult has been attributed to cytokine induction of acute phase protein release from liver, of which the 14 kDa-$PLA_2$ is suggested to be a part (Crowl, et al., (1991) J. Biol. Chem. 266, 2647-2651). In addition, soluble $PLA_2$ activity is markedly elevated in the serum and synovial fluid of patients with rheumatoid arthritis (Stefanski et al., J. Biochem. 100:1297-303 (1986). Furthermore, increasing serum $PLA_2$ levels have been shown to positively correlate with clinical severity (Bomalaski and Clark, Arthritis and Rheumat. 36:190-198 (1993)). Various inhibitors of $PLA_2$ have been described in publications and in US Patents. See for instance US Patents 4,959,357; 4,933,365; 5,208,223; 5,208244; Marshall et al., J. Rheumatology 18:1 (1991); Marshall et al., Phospholipase A2, Ed. Pyu Wong, Plenum Press, NY (1990) pages 169-181; Wilkerson, et al., Eur. J. Med. Chem., 26:667, 1991 and Wilkerson, Antiinflammatory Phospholipase $A_2$ Inhibitors, Drugs of the Future, Vol. 15, No. 2 p 139-148(1990). Accordingly, as $PLA_2$ is important in the liberation of arachidoninc acid from phospholipid and may also play a role in the generation of PAF via lysophospholipid formation, inhibition of such an enzyme would be useful for the treatment of disease states caused thereby.

There are many novel forms of phospholipase A2's which have recently been discovered. For the purposes herein, members of the sn-2 acylhydrolase family of PLA2's are divided into low and high molecular weight enzymes be it from mammalian, or non-mammalian sources. Low molecular weight $PLA_2$'s will generally have a molecular weight in the range of 12,000 to 15,000. High molecular weight will be in the range of 30,000 or 56,000 kDa to 110,000 by SDS electrophoresis analysis.

Preferred compounds for use herein as PLA2 inhibitors include those compounds of Formula (I) represented by the structure:



(I)

wherein

$R_1$ is $(CH_2)_nOH$ or $(CH_2)_nCO_2R_8$;

n is 0 or an integer a value of 1;

X is oxygen or sulfur;

$R_2$ is hydrogen, halogen, optionally substituted $C_{1-8}$ alkyl, or $C_{1-8}$ alkoxy;

m is 0 or an integer having a value of 1 or 2;

$R_3$ is $S(O)_2$ $R_7$;

R4 is hydrogen or $S(O)_2R_7$;

$R_5$ is hydrogen, halogen, $CF_3$, $CH_3$, $(CH_2)_tC(O)_2R_9$, or $(CH_2)_tOH$;

t is 0 or an integer having a value of 1 or 2;

$R_6$ is hydrogen or halogen;

R7 is optionally substituted aryl, optionally substituted aryl$C_{1-2}$ alkyl, or an optionally substituted $C_{1-8}$ alkyl;

R8 is hydrogen or $C_{1-4}$ alkyl;

R9 is hydrogen or $C_{1-4}$ alkyl;

or a pharmaceutically acceptable salt thereof.

$R_1$ is suitably $(CH_2)_nOH$ or $(CH_2)_nCO_2R_8$. Preferably $R_1$ is $(CH_2)_nCO_2R_8$ and n is preferably 0. R8 is preferably hydrogen or methyl, more preferably hydrogen, or a pharmaceutically acceptable salt thereof.

Suitably, $R_2$ is independently a substituent on the benzene ring from 1 to 2 times, and such substituent is selected from hydrogen, halogen, an optionally substituted $C_{1-8}$ alkyl, or $C_{1-8}$ alkoxy group Suitably when $R_2$ is halogen it is a chlorine, bromine, fluorine or iodine. When $R_2$ is an optionally substituted $C_{1-8}$ alkyl, the alkyl is substituted one to three times with halogen, such as fluorine, preferably a trifluoromethyl group. The optionally substituted $C_{1-8}$ alkyl moiety if preferably a branched $C_5$ chain, such as 1,1-dimethyl propyl moiety or a $C_8$ branched chain such as 1,1,3,3-tetramethyl butyl moiety.

Suitably, $R_3$ is $S(O)_2$ $R_7$; and R7 is an optionally substituted aryl, an optionally substituted aryl$C_{1-2}$ alkyl, or an optionally substituted $C_{1-8}$ alkyl group. Preferably when R7 is an aryl moiety it is phenyl or naphthyl, preferably phenyl;

when $R_7$ is an aryl alkyl moiety it is preferably benzyl. Suitably the aryl, aryl alkyl or alkyl moieties are substituted independently, one to three times, by halogen, trifluoromethyl, aryloxy, methoxy, $CH_2OH$, methyl, or $C(O)_2H$. Preferably, the substituents are halogen, or trifluoromethyl. The substituent halogen groups are preferably Cl, Br and fluorine. Preferably the substituents are in the 3,5- position or the 4-position of the aryl ring. More preferably the aryl substituents are 3,5-bis-trifluoromethyl, 4-trifluoromethyl, 4-bromo, 4-chloro, or 4-fluoro.

When $R_7$ is an optionally substituted alkyl moiety it is preferably a methyl or a $C_8$ unbranched chain. The methyl moiety, if substituted, is preferably substituted by one or more fluorines, such as in a trifluoromethyl group.

$R_4$ is suitably hydrogen or $S(O)_2R_7$. Preferably $R_4$ is hydrogen. When $R_4$ is $S(O)_2R_7$ the $R_7$ group is preferably the same $R_7$ moiety as in the $R_3$ group forming a bis structure.

Suitably $R_5$ is hydrogen, halogen, $CF_3$, $CH_3$, $CH_2C(O)_2R_9$, or $CH_2OH$, wherein t is 1. Preferably when $R_5$ is $CH_2C(O)_2R_9$, $R_9$ is a $C_{1-4}$ alkyl, preferably t-butyl. Preferred $R_5$ groups are hydrogen, $CF_3$, or halogen. More preferably $R_5$ is hydrogen or $CF_3$.

Suitably $R_6$ is hydrogen or halogen; preferably hydrogen. If $R_6$ is halogen it is preferably fluorine or chlorine.

Additional compounds for use herein as $PLA_2$ inhibitors are found in U.S. Patent 5,470,882, Dixon et al., granted November 28, 1995; WO95/33712, Dixon et al., published December 14, 1995; U.S. Patent 5,545,669, Adams et al., granted August 13, 1996; WO95/33461, Adams et al., published December 14, 1995; WO95/33462, Adams et al., published December 14, 1995; U.S. Patent 5,447,957, Adams et al., granted September 5, 1995; WO95/33460, Adams et al., published December 14, 1995; WO95/33458, Adams et al., published December 14, 1995; WO95/33713, Adams et al., published December 14, 1995; WO95/33715, Eggleston et al., published December 14, 1995; U.S. Patent 5,496,855, Adams et al., granted March 5, 1996; WO96/22770, Adams et al., published August 1, 1996.

Suitable pharmaceutically acceptable salts are well known to those skilled in the art and include basic salts of inorganic and organic acids, such as hydrochloric acid, hydrobromic acid, sulphuric acid, phosphoric acid, methane sulphonic acid, ethane sulphonic acid, acetic acid, malic acid, tartaric acid, citric acid, lactic acid, oxalic acid, succinic acid, fumaric acid, maleic acid, benzoic acid, salicylic acid, phenylacetic acid and mandelic acid. In addition, pharmaceutically acceptable salts of compounds of formula (I) may also be formed with a pharmaceutically acceptable

cation, for instance, if substituent $R_1$ comprises a carboxy group. Suitable pharmaceutically acceptable cations are well known to those skilled in the art and include alkaline, alkaline earth, ammonium and quaternary ammonium cations.

The following terms, as used herein, refer to:

- "halo" - all halogens, that is chloro, fluoro, bromo and iodo;
- "$C_{1-8}$ alkyl" or "alkyl" - both straight and branched chain radicals of 1 to 8 carbon atoms, unless the chain length is otherwise limited, including, but not limited to, methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl, *tert*-butyl, and the like;
- "aryl" - phenyl and naphthyl;
- "aryl alkyl" - is used herein to mean a phenyl and naphthyl connected to a $C_{1-4}$ alkyl as defined above unless otherwise indicated;

The compounds of the present invention may contain one or more asymmetric carbon atoms and may exist in racemic and optically active forms. All of these compounds are included within the scope of the present invention.

Specifically exemplified compounds of Formula (I) for use herein are:

2-[2-[3,5-bis(Trifluoromethyl)phenylsulfonamido]-4-trifluoromethylphenoxy]benzoic acid;

2-[2-(4-Bromophenylsulfonamide)-4-trifluoromethylphenoxy]benzoic acid;

2-[2-[3,5-Bis(trifluoromethyl)phenylsulfonamido]phenoxy]benzoic acid;

2-[2-(2-Naphthylsulfonamido)-4-trifluoromethylphenoxy]benzoic acid;

2-[2-(2-Naphthylsulfonamido)phenoxy]benzoic acid;

2-[2-[3,5-Bis(trifluoromethylphenyl)]sulfonamido-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzoic acid;

2-[2-(Octylsulfonamido)phenoxy]benzoic acid; (also referred to as 2-[2-[[(Octylsulfonyl)amino]phenoxy]benzoic acid);

2-[2-[3,5-Bis(trifluoromethyl)phenylsulfonamido]-4-methylphenoxy]benzoic acid;

2-[2-[[(Methylsulfonyl)amino]-4-(trifluoromethyl)phenoxy]benzoic acid;

2-[2-[[Octylsulfonyl)amino]-4-(trifluoromethyl)phenoxy]benzoic acid;

2-[2-[3,5-bis(Trifluoromethyl)phenyl-N-methylsulfonamido]-4-trifluoromethylphenoxy]benzoic acid;

2-[2-(Phenylsulfonamido)-4-trifluoromethylphenoxy]benzoic acid;

2-[2-(4-Chlorophenylsulfonamido)-4-trifluoromethylphenoxy]benzoic acid;

2-[2-(1-Naphthylsulfonamido)-4-trifluoromethyl-phenoxy]benzoic acid;

2-[2-(Phenylmethylsulfonamido)-4-trifluoromethylphenoxy]benzoic acid;

2-[2-(4-Trifluoromethylphenylsulfonamido)-4-trifluoromethylphenoxy]benzoic acid;

2-[2-[3,5-bis(Trifluoromethyl)phenylsulfonamido]-4-trifluoromethylphenoxy]phenylacetic acid;
2-[2-(4-Fluorophenylsulfonamido)-4-trifluoromethylphenoxy]benzoic acid;
2-[2-(4-Methoxyphenylsulfonamido)-4-trifluoromethylphenoxy]benzoic acid;
2-[2-[3,5-Bis(trifluoromethyl)phenylsulfonamido]-4-(trifluoromethyl)-phenoxy]-4-methoxybenzoic acid;
2-[2-[N,N-Bis[3,5-bis(trifluoromethyl)phenylsulfonyl]amino]-4-(trifluoromethyl)phenoxy]-4-methoxybenzoic acid;
2-[2-[N,N-Bis-[3,5-bis(trifluoromethyl)phenylsulfonyl]amino]-4-(trifluoromethyl)phenoxy]benzoic acid;
2-[2-[3,5-Bis(trifluoromethyl)phenylsulfonamido]-4-bromophenoxy]benzoic acid;
2-[2-[[[3,5-Bis(trifluoromethyl)phenyl]sulfonyl]amino]-4-bromo-phenoxy]benzoic acid;
2-[2-(4-Hydroxymethylsulfonamido)-4-trifluoromethylphenoxy]benzoic acid;
6-[2-[3,5-Bis(trifluoromethyl)phenylsulfonamido]-4-trifluoromethylphenoxy]-2-methoxybenzoic acid;
2-[2-[3,5-Bis(trifluoromethyl)phenylsulfonamido]-4-trifluoromethylthiophenoxy]benzoic acid;
2-[2-[3,5-Bis(trifluoromethyl)phenylsulfonamido]-4,5-dichloro-phenoxy]benzoic acid;
2-[2-[3,5-Bis(trifluoromethyl)phenylsulfonamido]-4-trifluoromethylphenoxy]benzyl alcohol;
2-[2-(4-Chlorophenylsulfonamido)-4,5-dichlorophenoxy]benzoic acid;
2-[2-(4-Bromophenylsulfonamido)-4-(carboxymethyl)phenoxy]benzoic acid;
2-[2-(4-Bromophenylsulfonamido)-4-(hydroxyethyl)phenoxy]benzoic acid;
Methyl 2-[2-(4-Bromophenylsulfonamido)-4-(carboxymethyl)-phenoxy]benzoate;
2-[2-(4-Bromophenylsulfonamido)-4-(tert-butoxycarbonylmethyl)-phenoxy]benzoic acid;
2-[2-[(Trifluoromethylsulfonyl)amino]-4-(trifluoromethyl)phenoxy]benzoic acid;
2 -[trans-2-[3,5-Bis(trifluoromethyl)phenylsulfonamido]cyclohexyloxy]benzyl alcohol; and
2 -[trans-2-[3,5-Bis(trifluoromethyl)phenylsulfonamido]cyclohexyloxy]benzoic acid.

In order to use a compound of formula (I) or a pharmaceutically acceptable salt thereof in therapy, it will normally be formulated into a pharmaceutical composition in accordance with standard pharmaceutical practice. This invention,

therefore, also relates to a pharmaceutical composition comprising an effective, non-toxic amount of a compound of formula (I) and a pharmaceutically acceptable carrier or diluent.

Compounds of formula (I), pharmaceutically acceptable salts thereof and pharmaceutical compositions incorporating such may conveniently be administered by any of the routes conventionally used for drug administration, for instance, orally, topically, parenterally or by inhalation. The compounds of formula (I) may be administered in conventional dosage forms prepared by combining a compound of formula (I) with standard pharmaceutical carriers according to conventional procedures. Such pharmaceutically acceptable carriers or diluents and methods of making are well known to those of skill in the art, and reference can be found in such texts as Remington's Pharmaceutical Sciences, 18th Ed., Alfonso R. Genarao, Ed., 1990, Mack Publishing Co. and the Handbook of Pharmaceutical Excipents, APhA Publications, 1986.

The compounds of formula (I) may also be administered in conventional dosages in combination with known second therapeutically active compounds, such as steroids or NSAID's for instance. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The pharmaceutical carrier employed may be, for example, either a solid or liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are syrup, peanut oil, olive oil, water and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl mono-stearate or glyceryl distearate alone or with a wax.

A wide variety of pharmaceutical forms can be employed. Thus, if a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form or in the form of a troche or lozenge. The amount of solid carrier will vary widely but preferably will be from about 25mg. to about 1g. When a liquid carrier is used, the preparation will be in the form of a syrup, emulsion, soft

gelatin capsule, sterile injectable liquid such as an ampule or nonaqueous liquid suspension.

Compounds of formula (I) may be administered topically, that is by non-systemic administration. This includes the application of a compound of formula (I) externally to the epidermis or the buccal cavity and the instillation of such a compound into the ear, eye and nose, such that the compound does not significantly enter the blood stream. In contrast, systemic administration refers to oral, intravenous, intraperitoneal and intramuscular administration.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of inflammation such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose. The active ingredient may comprise, for topical administration, from 0.001% to 10% w/w, for instance from 1% to 2% by weight of the formulation. It may however comprise as much as 10% w/w but preferably will comprise less than 5% w/w, more preferably from 0.1% to 1% w/w of the formulation.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy base. The base may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives or a fatty acid such as steric or oleic acid together with an alcohol such as propylene glycol or a macrogel. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surfactant such as a sorbitan esteror a polyoxyethylene derivative thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as silicaceous silicas, and other ingredients such as lanolin, may also be included.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 98-100 °C. for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Each dosage unit for oral administration contains preferably from 1 to 250 mg (and for parenteral administration contains preferably from 0.1 to 25 mg) of a compound of the structure (I) or a pharmaceutically acceptable salt thereof calculated as the free base.

The pharmaceutically acceptable compounds of the invention will normally be administered to a subject in a daily dosage regimen. For an adult patient this may be, for example, an oral dose of between 1 mg and 500 mg, preferably between 1 mg and 250 mg, or an intravenous, subcutaneous, or intramuscular dose of between 0.1 mg and 100 mg, preferably between 0.1 mg and 25 mg, of the compound of the Formula (I) or a pharmaceutically acceptable salt thereof calculated as the free base, the compound being administered from 1 to 4 times per day.

The choice of form for administration, as well as effective dosages, will vary depending, inter alia, on the condition being treated. The choice of mode of administration and dosage is within the skill of the art.

Chronic diseases which have an inappropriate angiogenic component are various ocular neovasularizations, such as diabetic retinopathy and macular degeneration.

Other chronic diseases which have an excessive or increased proliferation of vasculature are tumor growth and metastasis, atherosclerosis, and certain arthritic conditions. Therefore cytokine inhibitors will be of utility in the blocking of the angiogenic component of these disease states.

The term "excessive or increased proliferation of vasculature inappropriate angiogenesis" as used herein includes, but is not limited to, diseases which are characterized by hemangiomas and ocular diseases.

The term "inappropriate angiogenesis" as used herein includes, but is not limited to, diseases which are characterized by vesicle proliferation with accompanying tissue proliferation, such as occurs in cancer, metastasis, arthritis and atherosclerosis.

Described below a model of inflammatory angiogenesis is used to show that $PLA_2$ inhibition will stop the tissue destruction of excessive or inappropriate proliferation of blood vessels. Based on these observations, a $PLA_2$ inhibitor, 2-[2-[3,5-bis(Trifluoromethyl)-phenylsulfonamido]-4-trifluoromethylphenoxy]benzoic acid, Compound I, was tested for its ability to inhibit angiogenesis in an *in vivo* animal model of inflammatory angiogenesis.

The murine airpouch granuloma model of chronic inflammation (Kimura et al., 1985, J. Pharmacobio-Dyn., 8:393-400; Colville-Nash et al.,1995, J. Pharm. and Exp. Ther., 274:1463-1472) whose disclosure is incorporated herein by reference in its entirety, is characterized by inflammatory cell influx, fibrous tissue proliferation and intense angiogenesis. It is representative of inflammatory angiogenesis and demonstrates that the angiogenic component can be pharmacologically modulated independently of granuloma growth and size. In addition, angiogenesis can be accurately quantitated by a vascular casting method.

The effect of the compound on vascular density (and dry weight) was measured for 6 days after induction of the granuloma. This time point has been determined to be at or near the peak of angiogenesis. Compound I demonstrated a significant decrease in the vascular index with a maximum reduction of 36% at 30mg/kg, bid, i.p. As a positive control medroxyprogesterone, an angiostatic steroid (Gross et al., 1981, Proc. Natl. Acad. Sci. USA, 78:1176-1180) - whose disclosure is hereby incorporated by reference in its entirety, was utilized. This control demonstrated a maximum reduction of 50% in this model. Neither Compound I nor medroxyprogesterone had an effect on granuloma size as measured by dry weight.

Angiogenesis in the granuloma was microscopically evaluated. The vasculature of day 6 granulomas from both untreated and compound I treated mice was examined. The profound angiogenesis in the granuloma is demonstrated by the extensive vascular network in the control tissue. There was a striking reduction in the vasculature of the treated tissue. In fact, there was almost no fine capillaries visible, only a few larger vessels.

Prostanoids and leukotrienes are the downstream products of the arachidonic acid released by $PLA_2$. The levels of these lipid mediators in the granuloma were measured to determine if there modulation by compound I correlated with angiogenesis. Prostaglandin $D_2$ ($PGD_2$) and leukotriene $B_4$ ($LTB_4$) levels were measured by ELISA using homogenates of day 6 granuloma tissue. $LTB_4$ was measured at 3404 pg/ml in vehicle treated animals and 2042 pg/ml in compound I treated animals. Thus compound I reduced LTB4 levels by 40 %. In contrast, $PGD_2$ levels were not affected by compound I, 378 pg/ml $PGD_2$ in vehicle treated animals and 403 pg/ml $PGD_2$ in compound I treated animals. These results are consistent with specific inhibition of 14 kD $PLA_2$ by compound I in vivo, because 14 kD $PLA_2$ releases arachidonic acid for leukotriene synthesis, while arachidonic acid used for prostaglandin systhesis is released by 85 kD $PLA_2$.

**Methods:**

Murine air pouch granuloma model:

Day -1, mice are anesthetized using Aerrane (isoflurane) gas (5%), after which 3mls of air is injected into the dorsal subcutaneous tissue using a 27g needle. Mice are allowed to recover.

Day 0, mice are again anesthetized using Aerrane, once anesthetized 0.5ml of Freunds complete adjuvant with 0.1% v/v croton oil is injected into the air pouch formed on Day -1. The animals also begin their dosing regime (number of days dependent upon study) with the animals typically receiving compound in 0.2ml N,N, Dimethyl Acetoacetamide(DMA)(Sigma, St. Louis, Mo.)/Cremephor El (Sigma, St. Louis, Mo.)/ saline (10/10/80) or other appropriate vehicle. The animals are allowed to recover and all subsequent dosing is performed on the animals in the absence of anesthetics.

Days 1-5, animals are dosed according to schedule.

On Day 6 the animals are again anesthetized using Aerrane after which a vascular cast is made (Kimura et al., 1986, J.Pharmacobio-Dyn., 9:442-446), this involves a 1ml tail vein i.v. injection of a Carmine Red(10%)(Sigma, St. Louis, Mo.)/ gelatin (5%)(Sigma, St. Louis, Mo.) solution. The animals are then sacrificed by lethal dose of anesthesia and chilled at 4 C for 2 hours prior to the removal of the granuloma tissue.

When the granuloma is removed it is weighed and then dried for 3 days at 45 C and reweighed. The dried tissue is then digested in 0.9ml of a 0.05M phosphate

buffer pH 7.0 containing 12 U /ml$^{-1}$ papain (Sigma, St. Louis, Mo.) and 0.33g / L$^{-1}$ N-acetyl-l-Cysteine (Sigma, St. Louis, Mo.) at 57 C for 3 days. After 3 days digestion the carmine red is solubilized by the addition of 0.1ml 5mM NaOH. Samples are centrifuged and then filtered using 0.2um acrodiscs. The carmine content is then determined against a carmine red standard curve (0.5 to 2 mg/ml) generated in extracted tissue from non carmine treated animals and read at 490nm. Sample and standard values are determined using DeltaSoft Elisa analysis software (Biometallics Inc., Princeton, NJ). The carmine content is then used to determine the vascular indexes for the various treatments, vascular index being the mg carmine dye/gm dry tissue.

Tissue extracts were made by homogenizing granulomas in 0.5ml 5mM $KH_2PO_4$ / 0.1gm wet tissue. $PGD_2$ and $LTB_4$ were measured using ELISA kits from Cayman Chemical Company. Sample values for both ELISAs are calculated using DeltaSoft ELISA analysis software (Biomettalics Inc., Princeton, NJ).

**Figure 1** shows the tissue dry weight and vascular index (VI, mg carmine dye/ gm dry tissue) of Day 6 granulomas following treatment with compound I or medroxyprogesterone. Dosing began at the time granulomas were induced. VI data is expressed as a percent of vehicle treated control animals +/- S.D. Control VI=4.6. control dry weight=0.25 gms. n=5. * significant from control at $p<0.05$, calculated by Duncan's multiple range test.

To determine $PLA_2$ activity of the compounds for use herein various cellular assays can be used. Additionally, various classical in vivo acute inflammatory models which have some aspect of their etilogy to elevated eicosanoid levels can be employed, such as the paw edema model, mouse zymosan peritonitis, reverse Arthus pleurisy or various skin inflammation assays which are described in Lewis et al., Experimental Models of Inflammation, in the Handbook of Inflammation, Vol. 5, Bonta Ed., Elsevier Science Publishers, NY (1985) whose disclosure is herein incorporated by reference. The TPA induced ear edema model (mouse) as well as the carrageenan paw edema model in the rat are described herein as well. These classical models of inflammation will reflect the drug's ability to alter an inflammatory response but cannot address the specificity of drug action. These models have been traditionally designed as non steriod antiinflammatory drug sensitive pharmacological screens and it is important to utilize models which can differentiate $PLA_2$ from NSAIDS, such as described below.

**Phospholipase $A_2$ assay:**

Phospholipase $A_2$ activity of rh Type II- 14 kDa $PLA_2$ or $PLA_2$ semi-purified from human synovial joint fluid was measured by the acylhydrolysis of high specific activity (NEN)[$^3$H]-AA-*E. coli* (0.5 mCi/5nmol PL Pi) as previously described in Marshall et al., J. Rheumatology, 18:1, pp59-65 (1991). High specific activity [$^3$H]AA-*E. coli* had up to 95% of the label incorporated into phospholipid which was localized almost exclusively in the sn-2 position, as demonstrated by purified 14kDa PLA2 or low molecular weight $PLA_2$ acylhydrolysis and separation of products by thin layer chromatography (TLC) (data not shown). [Predominately used herein was rh Type II 14 kDa PLA2, or alternatively bovine pancreatic $PLA_2$ was also be used]. The reaction mixture (50 or 100 ml total volume) contained 25 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM $CaCl_2$ and [$^3$H]-AA-E. coli (low specific activity; 5-8 nmol PL Pi per assay). Assays were incubated for a time predetermined to be on the linear portion of a time versus hydrolysis plot. Experiments were conducted with final % hydrolysis values ranging from 2% (400-1000 dpm) to 10% (2000-5000 dpm) acylhydrolysis after blank correction. Reactions were terminated by the addition of 1.0 mL tetrahydrofuran (THF). The whole sample was placed over aminopropyl solid phase silica columns and eluted with THF:acetic acid (49:1) exclusively separating free fatty acids with greater than 95% recovery. Radiolabel in this eluate was quantitated by liquid scintillation counting. Results were expressed as % of fatty acid hydrolyzed ([sample dpms - non-specific (blank) dpms/total dpms] x 100) or specific activity which was calculated from hydrolysis values found in the linear portion of time versus % hydrolysis plots (pmol free fatty acid hydrolyzed/mg/min). Non-specific activity was always less than 1% of the total counts added.

**Protein Determination**

All protein concentrations were determined by Bradford protein analysis kits (Biorad, Richmond, CA). All of the exemplified compounds of Formula (I) described herein demonstrated positive $PLA_2$ inhibition in the method noted above. While these compounds generally tested positive at 50 μm levels, several were also tested for positive inhibitory activity at up to 500 μM levels.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.